STUDIES TOWARDS THE CHEMICAL SYNTHESIS OF THE 18 KDA ANTIGENIC PROTEIN FROM *MYCOBACTERIUM LEPRAE*.

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ABSTRACT

This thesis describes the solid phase synthesis of a series of peptides from the antigenic *Mycobacterium leprae* 18 kDa protein and the total synthesis of the 148 amino acid protein.

The peptides were synthesised using an Applied Biosystems 430A Solid Phase Peptide Synthesiser usually modified by the removal of in-line filters to the reaction vessel to allow the synthesis of the peptides using programs evolved by S.B.H. Kent. The peptides were cleaved from the peptide resin using liquid HF and purified by reverse phase HPLC.

The first series of peptides to be synthesised revolved around a monoclonal antibody binding site. These peptides were SLP-1, (101-115, RILASYQEGVLKLSI), SLP-2, (111-124, LKLSIPVAERAKPRK), SLP-3, (121-134, AKPRKISVDRGNNG) and SLP-4 (109-125 GVLKLSIPVAERAKPRK). The other peptides were synthesised as a series of overlapping 20 mers covering the entire 148 amino acid sequence. These peptides are SLP-5 (1-20, MLMRTDPFRELDRFAEQVLG), SLP-6 (16-35, EQVLGTS ARPAVMPMDAWRE), SLP-7 (31-50, DAWREGEFVVEFDLPGIKA), SLP-8 (46-65, PGIKADSLDIDIERNVVTVR), SLP-9 (61-80, VVTVRAERPGVDPDREMLAA), SLP-10 (76-95, EMLAAERPRGLFNRQLVLGE), SLP-11 (91-110, LVLGENLDTERIL ASYQEGV), SLP-12 (106-125, YQEGVLKLSIPVAERAKPRK), SLP-13 (121-140, AKPRKISVDRGNNGHQTINK) and SLP-14 (136-148, QTINKTAEHEIIDA). Two other peptides SLP-15 (101-125, RILASYQEGVLKLSIPVAERAKPRK) and SLP-16 (91-115, LVLGEIVLDTERILASVQEGVLKLSI) were also synthesised.

The peptides were used in immunological studies that determined the location of the L-5 monoclonal antibody binding site and showed where T-cell stimulation sites are located on the 18 kDa protein in murine systems.

The synthesis of larger 50 amino acid fragments of the 18 kDa protein, peptides SLP-17 (101-148), SLP-18 (50-100) and SLP-19 (1-50) was carried out in order to determine if it was possible to synthesise the total 18 kDa protein. These fragments were synthesised in a similar manner to the previous peptides. From the synthesis of these peptides it was decided that the total synthesis of the 148 amino acid protein was possible.

The synthesis of the 18 kDa protein was carried out using Kent's protocols as a single step process. The synthesis was monitored up to the 100th amino acid by ninhydrin assay with no failed couplings detected. The coupling percentages for all of the amino

acids was achieved by peptide resin sequencing where the average percentage couplings were shown to be 99.49% with an overall yield for the protein on the resin of 49%.

After Lo-Hi HF cleavage purification of the protein was hampered by the formation of aggregated products which proved initially to be inseparable from the protein. By sequencing some partially purified protein it was shown that under certain cleavage conditions the benzyl ether side chain protecting group was present on the threonine amino acids at positions 5 and 21. A further treatment of the protein using a hard acid-soft base mechanism with trimethylsilyl trifluorosulphonate/thioanisole was used in these cases to remove the remaining benzyl protecting groups.

An attempt to overcome the aggregation of the protein involved the addition of SDS to the HF cleavage vessel. After purification the protein showed no signs of the aggregation products.

A 90 amino acid fragment removed during the course of the synthesis of the 18 kDa proteien was cleaved and dissolved in 6M guanidine.HCl. After gel filtration on a Sephadex G-50 column, preparative HPLC was carried out on the isolated protein peak. The protein was then gel filtered again on a Sephadex G-50 column using 6M guanidine.HCl which separated the aggregated product to give a pure 90 amino acid protein after dialysis.

The full synthetic 18 kDa protein was purified in a similar manner to the 90 amino acid fragment with the second gel filtration being carried out using a Sephadex G-100 column or a Pharmacia Superose 12 column. This provided pure synthetic 18 kDa protein in an estimated 1.8 % yield or 47 mg from this synthesis of a final 2.46 g of protein resin based on the starting protein on the resin.

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